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CLAIMS

We claim:

1. A crystal of MurI complexed with an inhibitor.
2. A crystal of MurI complexed with an inhibitor and a substrate.
- 5 3. The crystal of claim 1, wherein the MurI is from a bacterium selected from a Gram positive bacterium, a Gram negative bacterium, and an atypical bacterium.
4. The crystal of claim 2, wherein the MurI is from a bacterium selected from a Gram positive bacterium, a Gram negative bacterium, and an atypical bacterium.
5. The crystal of claim 1, wherein the inhibitor is bound to a molecular
10 interface of MurI.
6. The crystal of claim 5, wherein the molecular interface of MurI is selected from the group consisting of a substrate binding site, an activator binding site, an intermolecular dimer interface, an intradomain interface, an inhibitor binding site, and a combination thereof.
- 15 7. The crystal of claim 6, wherein the MurI is *H. pylori* MurI.
8. The crystal of claim 7, wherein the substrate binding site of *H. pylori* MurI comprises amino acid residues: Ser8, Cys70, Thr72, Thr116, Thr119, Glu150, Cys181, Thr182, and His183 of SEQ ID NO: 2.
9. The crystal of claim 7, wherein the intermolecular dimer interface of *H.*
20 *pylori* MurI comprises amino acid residues: Ser34, Ala35, Arg36, Val37, Pro38, Tyr39, Gly40, Thr41, Lys42, Asp43, Pro44, Thr46, Phe50, Lys117, Asn121, Ser143, Leu144, Pro147, Leu148, Glu150, Glu151, Ser152, Ile153, Gly157, Leu158, Thr161, Cys162, Tyr165, Tyr166, Ser239, Gly240, Asp241, and Trp244 of SEQ ID NO: 2.

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10. The crystal of claim 7, wherein the intradomain interface comprises amino acid residues: Asp7, Ser8, Gly9, Val10, Gly11, Gly12, Phe13, Ser14, Val15, Ser18, Lys21, Ala22, Val37, Pro38, Tyr39, Gly40, Thr41, Lys42, Asp43, Pro44, Ile47, Ala69, Cys70, Asn71, Thr72, Ser74, Ala75, Leu76, Gly91, Val92, Gly211, Asp212, 5 Ala213, Ile214, Val215, Glu216, Tyr217, Leu218, Gln219, Gln220, Lys221, Glu251, Trp252, Leu253, Lys254, and Leu255 of a first domain, and amino acid residues Ile93, Glu94, Pro95, Ser96, Ile97, Leu98, Ala99, Ile100, Arg102, Gln103, Thr116, Lys117, Ala118, Thr119, Ser122, Asn123, Ala124, Tyr125, Ala128, Gln131, Gln132, Ser143, Val146, Pro147, Ile149, Glu150, Glu151, Ser152, Ile178, 10 Leu179, Gly180, Cys181, Thr182, His183, Phe184, Pro185, Leu186, Ile208, His209, Ser210, Gly211, and Asp212 of a second domain of SEQ ID NO: 2.
11. The crystal of claim 7 wherein the inhibitor binding site comprises amino acid residues Val10, Gly11, Phe13, Ile149, Glu151, Ser152, Leu186, Trp244, Gln248, and Trp252 of SEQ ID NO: 2.
- 15 12. The crystal of claim 6, wherein the MurI is *E. coli* MurI.
13. The crystal of claim 12, wherein the substrate binding site of *E. coli* MurI comprises amino acid residues: Ser29, Cys92, Thr94, Thr135, Thr138, Glu170, Cys204, Thr205, His206, and Phe207 of SEQ ID NO: 40.
- 20 14. The crystal of claim 12, wherein the activator binding site of *E. coli* MurI comprises amino acid residues: Thr97, Leu100, Pro101, Ala102, Arg104, Glu105, Lys106, Phe107, Asp108, Phe109, Pro110, Val111, Val112, Gly113, Val114, Val115, Pro116, Ala117, Ile118, Lys119, Pro120, Arg123, Leu124, Ser142, Tyr143, Thr144, Glu146, Leu147, Arg150, Phe151, Asp226, Ser227, Gly228, Ala229, Ala230, Ile231, Arg233, Arg234, Trp237, Leu238, Glu240 and His241 of SEQ ID 25 NO: 40.
15. The crystal of claim 12, wherein the intradomain interface of *E. coli* MurI comprises amino acid residues: Asp28, Ser29, Gly30, Val31, Gly32, Gly33, Leu34, Ser35, Val36, Asp38, Glu39, His42, Leu43, Val56, Ala57, Phe58, Pro59, Tyr60,

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Gly61, Glu62, Lys63, Ser64, Glu65, Ala66, Phe67, Ile68, Ala91, Cys92, Asn93, Thr94, Ala95, Ser96, Thr97, Val98, Val114, Ala230, Ile231, Ala232, Arg233, Arg234, Trp237, Leu238, Pro261, Gly262, Gln265, Leu266, Pro268, Val269, Leu270, Arg272, and Tyr273 of a first domain, and amino acid residues Val115,
5 Pro116, Ala117, Ile118, Lys119, Pro120, Ala121, Arg123, Leu124, Thr135, Arg136, Gly137, Thr138, Val139, Lys140, Arg141, Ser142, Tyr143, Thr144, Glu146, Leu147, Arg150, Ser162, Ala163, Val166, Gly167, Ala169, Glu170, Ala171, Lys172, Leu173, His174, Val201, Leu202, Gly203, Cys204, Thr205, His206, Phe207, Pro208, Leu209, Leu210, Val225, Asp226, Ser227, Gly228, and
10 Leu229 of a second domain, wherein the amino acid residues are represented by SEQ ID NO: 40.

16. The crystal of claim 6, wherein the MurI is *E. faecalis* MurI.

17. The crystal of claim 16, wherein the substrate binding site of *E. faecalis* MurI comprises amino acid residues: Ser12, Cys74, Thr76, Thr118, Thr121,
15 Glu153, Cys185, Thr186, and His187 of SEQ ID NO: 44.

18. The crystal of claim 16, wherein the intermolecular interface of *E. faecalis* MurI comprises amino acid residues: Gln26, Leu27, Pro28, Asn29, Glu83, Lys86, Ala87, Ala88, Leu89, Pro90, Ile91, Pro92, Val93, Val94, Gly95, Val96, Ile97, Leu98, Pro99, Arg102, Ala103, Lys106, Ala130, Ser133, Lys134, Ala135, Pro136,
20 Ala210, Glu211, Gly214, Glu215, Glu216, Ser217, Met218, Leu219, Asp221, Tyr222, Phe223, Asp224, Ile225, Ala226, His227, Thr228, and Pro229 of SEQ ID NO: 44.

19. The crystal of claim 16, wherein the intradomain dimer interface of *E. faecalis* MurI comprises amino acid residues: Asp11, Ser12, Gly13, Val14, Gly15,
25 Gly16, Leu17, Thr18, Val19, Lys21, Glu22, Lys25, Ala39, Arg40, Cys41, Pro42, Tyr43, Gly44, Pro45, Arg46, Pro47, Val51, Ala73, Cys74, Asn75, Thr76, Ala79, Val80, Val96, Ile97, Glu211, Thr212, Gly214, Glu215, Met218, Leu219, Asp221, and Tyr222 of a first domain, and amino acid residues Ile97, Leu98, Pro99, Gly100, Ala101, Arg102, Ala103, Ala104, Val105, Lys106, Val107, Thr118, Leu119,

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Gly120, Thr121, Lys123, Ser124, Ala125, Ser126, Tyr127, Ile129, Ala130, Ser133, Lys134, Cys145, Pro146, Lys147, Phe148, Val149, Pro150, Ile151, Val152, Glu153, Ser154, Asn155, Ile182, Leu183, Gly184, Cys185, Thr186, His187, Tyr188, Pro189, Leu190, Ile206, Asp207, Ser208, Gly209, and Ala210 of a second
5 domain, wherein the amino acid residues are represented by SEQ ID NO: 44.

20. The crystal of claim 6, wherein the MurI is *E. faecium* MurI.

21. The crystal of claim 20, wherein the substrate binding site of *E. faecium* MurI comprises amino acid residues: Ser15, Cys77, Thr79, Thr121, Thr124, Glu156, Cys188, Thr189, and His190 of SEQ ID NO: 48.

10 22. The crystal of claim 20, wherein the intermolecular interface of *E. faecium* MurI comprises amino acid residues: Gln29, Leu30, Pro31, Asn32, Glu86, Lys89, Ala90, Ala91, Leu92, Ser93, Ile94, Pro95, Val96, Ile97, Gly98, Val99, Ile100, Leu101, Pro102, Arg105, Lys109, Glu136, Lys137, Val138, Pro139, Glu214, Gly217, Glu218, Ser220, Met221, Leu222, Asp224, Tyr225, Phe226, Asn230,
15 Ser231, and Pro232 of SEQ ID NO: 48.

23. The crystal of claim 20, wherein the intradomain dimer interface of *E. faecium* MurI comprises amino acid residues: Asp14, Ser15, Gly16, Val17, Gly18, Gly19, Leu20, Thr21, Val22, Glu25, Lys28, Gln29, Arg43, Cys44, Pro45, Tyr46, Gly47, Pro48, Arg49, Pro50, Ala51, Val54, Ala76, Cys77, Asn78, Thr79, Ala82,
20 Val83, Val99, Glu214, Thr215, Val216, Gly217, Glu218, Met221, Leu222, Leu249, Phe250, Glu252, Ile253, Asp256, and Trp257 of a first domain, and amino acid residues Ile100, Leu101, Pro102, Gly103, Thr104, Arg105, Ala106, Ala107, Val108, Arg109, Lys110, Thr121, Ile122, Gly123, Thr124, Ser127, Gln128, Ala129, Tyr130, Leu132, Ala133, Leu134, Gly136, Lys137, Pro149, Lys150, Phe151,
25 Val152, Val155, Glu156, Ser157, Asn158, Ile185, Leu186, Gly187, Cys188, Thr189, His190, Tyr191, Pro192, Leu193, Ile209, Asp210, Ser211, Gly212, and Ala213 of a second domain, wherein the amino acid residues are represented by SEQ ID NO: 48.

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24. The crystal of claim 6, wherein the MurI is *S. aureus* MurI.
25. The crystal of claim 24, wherein the substrate binding site of *S. aureus* MurI comprises amino acid residues: Ser10, Cys72, Thr74, Thr116, Thr119, Glu151, Cys184, Thr185 and His186 of SEQ ID NO: 46.
- 5 26. The crystal of claim 24, wherein the intermolecular interface of *S. aureus* MurI comprises amino acid residues: Gln24, Leu25, Pro26, Asn27, Glu81, Glu84, Ser90, Val91, Ile92, Glu96, Pro97, Arg100, Thr101, Met104, Arg131, Ile132, Asn133, Pro134, Arg213, Glu214, Ser216, Ala217, Leu218, Thr220, Phe221, Ala226, Ser227, and Tyr228 of SEQ ID NO: 46.
- 10 27. The crystal of claim 24, wherein the intradomain dimer interface of *S. aureus* MurI comprises amino acid residues: Asp9, Ser10, Gly11, Val12, Gly13, Gly14, Leu15, Thr16, Val17, Glu20, Cys39, Pro40, Tyr41, Gly42, Pro43, Arg44, Pro45, Gly46, Val49, Ala71, Cys72, Asn73, Thr74, Ala77, Val78, Val94, Ile95, Glu210, Thr211, Ala212, Arg213, Glu214, Ala217, Leu218, His244, Asn247, Ile248,
- 15 Glu251, and Trp252 of a first domain and amino acid residues Ile95, Glu96, Pro97, Gly98, Ala99, Arg100, Thr101, Ala102, Ile103, Met104, Thr105, Thr116, Glu117, Gly118, Thr119, Ser122, Glu123, Ala124, Tyr125, His128, Arg131, Ile132, Pro144, Gly145, Phe146, Val147, Val150, Glu151, Gln152, Met153, Ile181, Leu182, Gly183, Cys184, Thr185, His186, Tyr187, Pro188, Leu189, Ile205, Ser206, Ser207,
- 20 Gly208, and Leu209 of a second domain, wherein the amino acid residues are represented by SEQ ID NO: 46.
28. A computer-assisted method of identifying an agent that is an inhibitor of MurI, comprising:
- (a) providing a computer modeling application with a set of relative
- 25 structural coordinates of a crystal of MurI, or of a molecular interface thereof;

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(b) supplying the computer modeling application with a set of structural coordinates of an agent to be assessed to determine if it binds a molecular interface of MurI;

(c) comparing the two sets of coordinates and;

5 (d) determining whether the agent is expected to bind a molecular interface of MurI;

wherein if the agent is expected to bind a molecular interface of MurI, an agent that is an inhibitor of MurI activity has been identified.

29. The computer-assisted method of claim 28, wherein the set of structural
10 coordinates are any one of Figures 4-19.

30. A computer-assisted method for designing an inhibitor of MurI activity, comprising:

(a) supplying to a computer modeling application a set of relative structural coordinates of MurI, or a molecular interface thereof;

15 (b) computationally building an agent represented by a set of structural coordinates; and

(c) determining whether the agent is expected to interfere with MurI, or a molecular interface thereof,

20 wherein if the agent is expected to interfere with the MurI or a molecular interface thereof, an inhibitor has been designed.

31. The computer-assisted method of claim 30, wherein the set of structural coordinates are any one of Figures 4-19.

32. A method of identifying a molecule that binds to MurI comprising:

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(a) applying a 3-dimensional molecular modeling algorithm to the atomic coordinates of a molecular interface of MurI; and

5 (b) electronically screening the stored spatial coordinates of a set of candidate compounds against the spatial coordinates of the molecular interface of MurI to identify compounds that bind to the molecular interface of MurI.

33. The method of claim 32, wherein the set of spatial coordinates are any one of Figure 4-19.

10 34. A method for structure-based design of a compound that fits a conserved surface of MurI comprising:

(a) producing a computer-generated representation of a conserved surface of a crystalline form of MurI;

(b) producing a computer-generated representation of a library of compounds to be assessed for their ability to fit the conserved surface; and

15 (c) determining if the compounds from step (b) fit the conserved surface,

wherein the crystalline form of the MurI is suitable for use in X-ray studies.

35. The method of claim 34, wherein the conserved surface of a crystalline form of MurI is represented by the relative structural coordinates of any of Figures 4-19.

20 36. A method of assessing the *in vitro* binding of a MurI inhibitor identified in a computer-assisted method comprising the steps of :

(a) culturing a test culture comprising a MurI substrate, the inhibitor, and a bacterium having MurI;

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(b) culturing the test culture for a period of time such that the bacterium having MurI in the culture expand; and

(d) comparing growth of the test culture to growth of an appropriate control culture,

5 whereby if growth of bacterium having MurI in the test culture is inhibited, an agent that binds MurI has been identified, wherein the agent that binds MurI is an inhibitor.

37. The method of claim 36, wherein the control culture is cultured simultaneously with the test culture.

10 38. The method of claim 36, wherein the control culture is cultured prior to the test culture.

39. The method of claim 36, wherein the control culture is cultured after the test culture.

15 40. The method of any of claims 28-39, further comprising testing the agent that binds MurI to determine if it inhibits MurI comprising the steps of:

(a) preparing a test culture of a substrate, a test inhibitor, and an atypical bacterium;

(b) preparing a control culture of a substrate and an atypical bacterium;

20 (c) culturing the atypical bacterial cultures for a period of time such that the atypical bacterium in the control culture expand; and

(d) comparing the growth of the control atypical bacterium to the growth of the test culture atypical bacterium,

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whereby if atypical bacterial growth in the test culture is inhibited, an agent that binds MurI has been identified, wherein the agent that binds MurI is an inhibitor.

41. A method for conducting a pharmaceutical business comprising:
- 5 a. isolating one or more MurI inhibitors that bind to MurI expressed in bacteria;
 - b. generating a composition comprising a MurI inhibitor, which composition inhibits growth with a minimal inhibitory concentration of 8 $\mu\text{g/mL}$ or less;
 - 10 c. conducting therapeutic profiling of the composition, for efficacy and toxicity in animals;
 - d. preparing a package insert describing the composition for treatment of bacterial infections; and,
 - e. marketing the composition for treatment of bacterial infections.
42. A method for conducting a life science business comprising:
- 15 a. isolating one or more MurI inhibitors that bind to MurI expressed in bacteria;
 - b. generating a composition comprising a MurI inhibitor, which composition kills with a minimal inhibitory concentration of 8 $\mu\text{g/mL}$ or less bacteria;
 - 20 c. licensing, jointly developing or selling, to a third party, the rights for selling the composition.
43. A method for conducting pharmaceutical business comprising:
- a. isolating one or more MurI inhibitors that bind to MurI expressed in bacteria with a K_d of 1 μM or less;

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- b. generating a composition comprising said MurI inhibitors, which composition has a minimal inhibitory concentration of 8 $\mu\text{g/mL}$ or less;
 - c. conducting therapeutic profiling of the composition for efficacy and toxicity in animals;
 - d. preparing a package insert describing the use of the composition for antibacterial therapy; and,
 - e. marketing the composition for use as an antibacterial agent.
- 44. A method for conducting a life science business comprising:
 - a. isolating one or more MurI inhibitors that bind to MurI expressed in bacteria with a K_d of 1 μM or less;
 - b. generating a composition comprising said MurI inhibitors, which composition has a minimal inhibitory concentration of 8 $\mu\text{g/mL}$ or less;
 - c. licensing, jointly developing or selling, to a third party, the rights for selling the composition.
- 45. A method of treating a bacterial infection comprising administering a MurI inhibitor.
- 46. Use of a MurI inhibitor for the treatment of a bacterial infection.
- 47. A pharmaceutical package comprising a composition of a MurI inhibitor and a pharmaceutically acceptable excipient labeled for the use of treating bacterial infections and instructions for use of the composition.